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STUDIES ON THE EXCRETORY PRODUCTS OF PEBRINE INFECTED TASAR SILKWORM, ANTHERAEA MYLITTA DRURY (DABA TV)

G. RENUKA AND G. SHAMITHA*

Department of Zoology, Kakatiya University, Warangal-506001, Andhra Pradesh, India

ABSTRACT

The tasar silkworm *Antheraea mylitta* Drury, is an important sericigenous insect reared outdoor and it is commercially exploited in 9 states of tropical India. , Pebrine is a disease caused in tasar silkworms, by a parasitic microsporidian- *Nosema mylittensis* leading to slow growth, undersized body and extreme crop loss. As excretion forms an important factor for the balance of nitrogen in the body, the present studies gives detail of the excretory products from fifth instar Pebrine-free and pebrine infected Tasar silkworm, *Antheraea mylitta* Drury (Daba TV)., The main excretory products like urea, uric acid, glutamine and ammonia were at a higher level in Pebrine-free worms as compared to Pebrine infected Tasar silkworms.

KEY WORDS: ***Antheraea mylitta,*** **excretory products, Pebrine, Tasar silkworm.**

G. SHAMITHA
Department of Zoology, Kakatiya University, Warangal-506001, Andhra Pradesh, India

Corresponding author

INTRODUCTION

The tasar silkworm *Antheraea mylitta* Drury is a species widely distributed in India between the range of 16 - 24^0 N latitude and 80 – 88^0 E from West Bengal in the East to Karnataka in the South with its natural inhabitation in the forest areas of Jharkhand, Bihar, Orissa, Madhya Pradesh, Maharashtra and Andhra Pradesh[1]. It is distributed in the form of about 43 ecoraces in varied geographical tropical zones in India[2]. It is trivoltine reared thrice a year in July – August (rainy cocoon crop), September – October (autumn cocoon crop) and November – December (winter crop). The silkworm is polyphagous feeding on a number of food plants of which Asan (*Terminalia tomentosa*. W& A.), Arjun (*T. arjuna*W&A.) and Sal (*Shorea robusta* gaertn.) are considered primary and many other secondary food plants[3,4] . It is a polyphagus insect feeding on number of food plants but primarily existing on *Terminalia arjuna, Terminalia tomentosa* and *Shorea robusta* and a host of other secondary food plants.

In the silkworm larva, nitrogenous waste products of metabolism are mainly excreted as urine together with faeces. The quantity of each nitrogenous compound in urine varied according to the food conditions during the fifth larval instar and also differed between silkworm races[5]. The excretion of nitrogenous waste products has been studied earlier in a number of insects [6,7]. In the silkworm larva, the nitrogenous waste products of metabolism are mainly excreted as urine, together with fecal pellets. The excretory pattern depends upon a number of environmental factors such as temperature and humidity[8,9]. The excretory pattern of silkworm larvae on exposure to F2 alpha increased the nitrogenous end products[10]. Similarly larvae feeding with trace elements like cobalt increased the pattern of excretion[11].

The diseases in silkworm are the major constraints in tasar culture, which adversely affect the economics of this culture by causing 35-40% crop loss. Among the diseases, pebrine is causing most devastating effect on the rearing of the tasar silkworm accounting for 20-25% loss in crop yield. [12.] Even though some work has been done on the breeding aspects of Tasar silkworm, not much work has been published so far on pathological aspects of tasar silkworm[13].

Pebrine is a disease caused in tasar silkworms, by a parasitic microsporidian-*Nosema mylittis,* belonging to the family Nosematidae, affected by which, the larvae exhibit slow growth, undersized body and poor appetite. Diseased larvae reveal pale and flaccid body. Tiny black spots appear on larval integument. Dead larvae remain rubbery and do not undergo putrefaction shortly after death The pathogen comes from infected eggs laid by infected mother moths may exist in rearing facilities as spores comes from wild insects naturally infected with *Nosema mylittis.*

Figure:1A

Uninfected Tasar silkworm, Antheraea mylitta D (Daba TV)

Figure:1B.
Pebrine infected tasar silkworm, Antheraea mylitta D (Daba TV) showing peppery integument

Excretion forms an important factor for the balance of nitrogen in the body. As there is limited information regarding tasar silkworm excretion, an attempt has been made here to compare the excretory pattern in the pellets of pebrine free and pebrine infected Tasar silkworm, *Antheraea mylitta* Drury (Daba TV).

MATERIALS AND METHODS

The trivoltine tasar silkworm, *Antheraea mylitta (Daba TV)* were reared in outdoor under normal condition on a diet of fresh *Terminalia arjuna* plantation, at the experimental garden of Kakatiya University at 26-33°C and 37-50% R.H and 13L+11D photoperiodic condition and , about two dfls (disease free layings) were reared during December 2011-February 2012. Two dfls of Pebrine free larvae were also collected from neighbouring forest patches of wild silkworm, *A.mylitta* (Daba TV). The incidence of Pebrine disease in the rearing lot was identified by the symptoms in the early larval stages. The appearance of black spots on the body and unequal larval development are the most prominent symptoms of this disease. They exhibit poor appetite resulting into stunted growth and irregular moulting. The larvae detected with Pebrine infection are immediately removed and buried far away from the rearing site. The instar-wise larval mortality is presented in the results (Table 1).

Table 1
Instar-wise Average Temperature (°C), Average Relative Humidity (%) & Mortality due to Pebrine disease of Tasar silkworm, Antheraea mylitta, (Daba TV).

Instar	Temperature©	Humidity(%)	Mortality	% Loss	Larval life span	
					Pebrine free	Pebrine infected
I	33.02 ± 0.61	37.6 ± 1.51	-	-		
II	33.8 ± 1.47	39 ± 2.30	10	4 %	4	5
III	33.05 ± 3.64	41.28 ± 2.42	51	20.4 %	4	4
IV	26.67 ± 0.51	49.55 ± 9.98	62	24.8 %	6	7
V	29.03 ± 2.50	45.43 ± 6.76	117	93.6 %	7	9

The values are expressed in terms of Standard Error of the Mean

The larvae soon become pale and dull with wrinkled cuticle. As the larval stages advance, the appearance of black spots in the integument becomes more prominent, exhibiting melanosis. The infected larvae (pebrine diseased) are detected by a method derived from that used in sericulture[14].

The excretory pellets of fifth instar larvae were collected separately and homogenized in precooled mortar and pestle thoroughly according to the measurements given in the following procedures. The contents are poured into centrifuge tubes. After centrifugation, the supernatant was collected into the test tubes and kept in deep freezer for subsequent use for biochemical estimations.

Centrifugation was done by using Remi centrifuge R-24 model. Most of the estimations based on calorimetric principle of Beer-Lambert's Law in which the absorbance of coloured complexes are proportional to the concentration of reaction products. The data was statistically analysed and the results have been discussed by comparing pebrine-free and pebrine-infected silkworms of *Antherea mylitta Drury*, (Daba TV).

Estimation of ammonia was done by using the method followed by Bergmeyer [15]. using nessler's reagent. Estimation of Glutamine was done by Kaplan (1957). method using Nessler's reagent. Estimation of Uric acid by the method described by Brocon and Oser [16,17]. Estimation of Urea is done by the method [18] described by Natelson.

Figure 2
Excretory pellets of tasar silkworm, Antheraea mylitta D(Daba TV)

RESULTS

The instar-wise average temperature and its standard deviation of *Antheraea mylitta* (DabaTV) rearing of 2011- 2012 were 33.02 ± 0.61 (S. D), 33.8 ± 1.47 (S. D), 33.05 ± 3.64 (S. D), 26.67 ± 0.51 (S. D) and 29.03 ± 2.50 (S. D) while that of rearing were I, II, III, IV and V instar respectively.

The instar-wise average relative humidity and its standard deviation of *Antheraea mylitta* (Daba TV) rearing of 2011-2012 were 37.6 ± 1.51 (S. D), 39 ± 2.30 (S. D), 41.28 ± 2.42 (S. D), 49.55 ± 9.98 (S. D) and 45.43 ± 6.76 (S. D) while that of rearing were I, II, III, IV and V instar respectively.

Table 2
Quantity of excretory products in fifth instar Antheraea mylitta Drury (Daba TV) expressed in mg.

Excretory Product	Pebrine-free	Pebrine-infected	Percentage difference/decrease
Ammonia	1.38 ±0.07	0.80±0.06	42.02
Glutamine	0.49 ±0.02	0.37±0.01	24.48
Urea	1.4± 0.02	0.90± 0.05	35.71
Uric acid	0.65±0.10	0.28±0.07	56.92

The values are expressed in terms of Standard Error of the Mean.

The ammonia content in the excretory pellets in the fifth instar of pebrine-free and pebrine-infected silkworms of *Antherea mylitta Drury*, (Daba TV) larvae were 1.38 ±0.07(S.D) and 0.80±0.06(S.D) mg per 50 mg of the excretory pellets respectively. The ammonia content in the Pebrine infected larvae has decreased to the extent of 42.02%

The content of glutamine in excretory pellets of pebrine-free and pebrine-infected silkworms of *Antherea mylitta Drury*, (Daba TV) larvae in fifth instar were 0.49 ±0.02 (S.D) and 0.37±0.01(S.D) mg per 50 mg of the excretory pellets respectively. It is observed that there is a huge decrease of 24.48%of glutamine in the Pebrine infected worms.

The content of Urea in excretory pellets of pebrine-free and pebrine-infected silkworms of *Antherea mylitta Drury*, (Daba TV) larvae in fifth instar were 1.4± 0.02 (S.D) and 0.90± 0.05(S.D) mg per 50 mg of the excretory pellets respectively. The extent of decrease of urea content in the Pebrine infected worms was about 35.71%.

The Uric acid content in the excretory products of fifth instar of pebrine-free and pebrine-infected silkworms of *Antherea mylitta Drury*, (Daba TV) larvae wee 0.65±0.10 (S.D) and 0.28±0.07(S.D) mg per 50 mg of the excretory pellets respectively and a decrease of 56.92% of uric acid was found in the Pebrine infected worms.

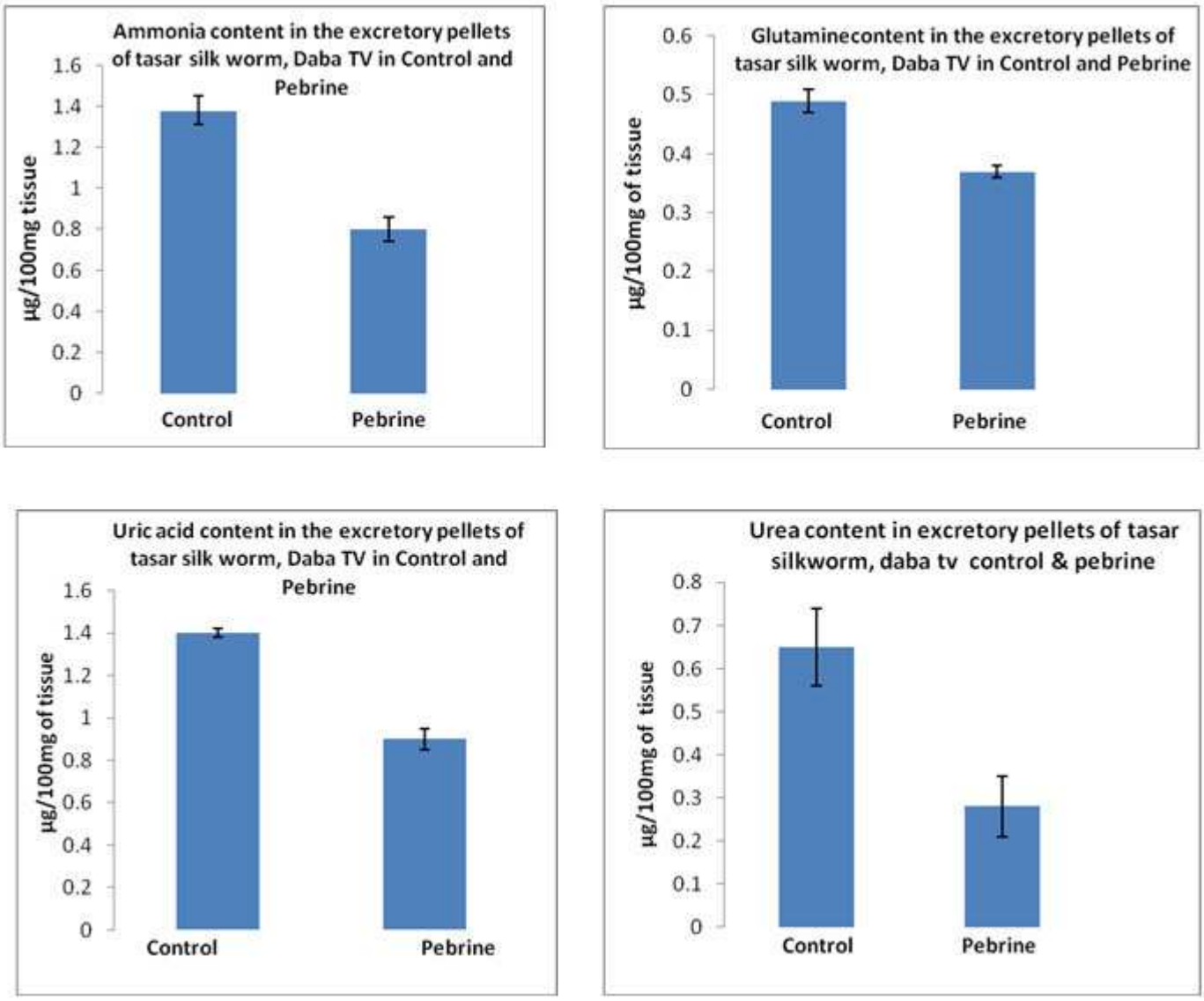
Table 3
Larval weight ,Silk gland Weight and % Decrease of Pebrine free and Pebrine infected V-instar Antheraea mylitta (Daba TV).

Sl. No	*Antherea mylitta Drury*, (Daba TV)	PEBRINE-free of *Antherea mylitta Drury*, (Daba TV)	PEBRINE- infected *Antherea mylitta Drury*, (Daba TV)	% Decrease
1.	Larval weight	22.48 ± 1.54	19.93 ± 1.80	11.34 %
2.	Silk gland weight	1.05 ± 0.04	0.40 ± 0.26	61.90 %

The average larval weight and its standard deviation of Pebrine free tasar silkworm, *Antheraea mylitta* Drury, was 22.48 ± 1.54 (S.D) while that of, Pebrine infected one was 19.93 ± 1.80 (S.D).

The average Silk gland weight and its standard deviation of Pebrine free tasar silkworm, *Antheraea mylitta* Drury, was 1.05 ± 0.04 (S.D) while that of Pebrine infected larva was 0.40 ± 0.26 (S.D).

Figure 3
Quantity of excretory products in fifth instar Antheraea mylitta Drury (Daba TV) expressed in mg.



DISCUSSION

The excretory products *viz.,* urea, uric acid, glutamine and ammonia were found to be at a higher level in fifth instar, normal/ Pebrine free tasar silkworm, *Antherea mylitta* D (Daba TV) when compared to the pebrine infected worms. Of the four nitrogenous products, urea is found to be of the maximum in both normal and Pebrine infected worms. The excretory pattern depends upon a number of environmental factors such as temperature and humidity[8,19]. It is also reported that excretion in insects is variable depending upon the habitat[20,21].

A few workers have attempted to demonstrate the excretory products of genus *Antheraea*[22,23]. Razet in 1961[24] has investigated the excretory metabolism of lepidopteran larvae at different stages of larval life. He concluded that there is a lability within the life history of the individual where the ratio of the major three end products may fluctuate enormously from day to day and the major end product is invariably the uric acid. Occurrence of uric acid as the main end product of nitrogen metabolism among insects is well established[25,26,27]. Thus insects are considered to be predominantly uricotelic in the classification of excretory metabolism[28]. It is an adaptation to terrestrial mode of life to overcome the shortage of water by excreting toxic end products like urea. However, there are few exceptions where the nitrogenous end products are largely other than uric acid in the excreta of variety of insects. A study on the excretory products of tasar silkworm, *Antherea mylitta* D (Andhra local ecorace) has also indicated an increased urea content in the fifth instar of outdoor and indoor reared worms[29].

This study suggests that there is an urgent need to develop an efficient method to control the disease. Although medium temperature and relative humidity can stimulate the pathogen [30.] some studies have shown that high temperature and relative humidity can cause minimum incidence of pebrine[31]. *Nosema mylittensis* is a well known entomopathogen of pebrine and loss incurred due to this disease varies from 35 −40% [12] . Some studies have indicated that[32] bleaching powder, slaked lime and formalin were effective in the inactivation of both *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV) and spores of *N. mylittensis,* while a recent report has revealed the indoor rearing performed at lower temperature and disinfected conditions can minimize the Pebrine to some extent[33].

In the present investigation, of the four nitrogenous excretory products in the fifth instar Pebrine free and Pebrine infected tasar silkworm, urea content is found to be maximum. It is also observed that there was a heavy loss of worms in the fifth instar. (Table-1). The studies also shows an enormous percentage of decrease in the uric acid content of Pebrine infected larvae, followed by ammonia, urea and glutamine (Table 2). It was also observed that the larval span has increased in the Pebrine infected larvae without corresponding increase in the body weight and silkgland weight (Table-3), resulting in crop loss and narrow prospects of silk production. During fifth instar the formation of nitrogenous products lessens due to depleted growth rate while silk synthesis in the glands takes place at a rapid pace. From the present studies it is clear that the content of all the major excretory products *viz.,* uric acid, urea, ammonia and glutamine in the Pebrine infected larvae has been decreased, with a corresponding decrease in larval and silkgland weight which may be due to poor nutrition coupled with lesser enzyme activity in the malphigian tubule and midgut which are the main regions involved in elimination of excretory material. The comparatively higher level of nitrogenous products in the uninfected silkworms may be due to higher breakdown of the end products which is correlated with the growth rate. As excretion forms an important

factor for the balance of nitrogen and water in the body and pebrine infection is found to be one of the major constraints in tasar culture, which causes heavy loss to the crops , the present study provides an avenue to explore the pathogenic effects of microsporidian-*Nosema mylittis* and pave way for production of quality silk.

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